Susceptibility to insecticides and enzymetic characteristics in the parasitoid *Apanteles plutellae* Kurdj.(Hymenoptera: Braconidae) and its host *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae)

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Abstract: The susceptibility to insecticides in the larval parasitoid *Apanteles plutellae* Kurdjumov (Hymenoptera: Braconidae) and its host *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), collected in Fuzhou, China, were detected using residual film and leaf-dip bioassays, respectively. The results showed that organophosphates, carbamates, pyrethroids, avermectins and fipronil were highly toxic to *A. plutellae*, but chlorfluazuron and Bt were not. However, *A. plutellae* could survive from the conventional control doses of fipronil, fenvalerate, cypermethrin and acephate if the parasitoid was left in contact with the insecticides only for short time (1 h). In *A. plutellae*, there were obvious synergisms of piperonyl butoxide (PB), triphenyl phosphate (TPP) and diethyl maleate (DEM) on methamidophos, carbofuran, fenvalerate, cypermethrin, avermectins and fipronil, but no synergisms on chlorfluazuron were found. The synergism of PB was the highest. Acetylcholinesterase (AChE) activity could not be inhibited by PB, TPP and DEM, but strong inhibition could be found in carboxylesterase (CarE) activity by PB and TPP, and in glutathione-S-transferase (GST) activity by DEM, *in vivo*. The apparent Michaelis-Menten constant (K_m), the maximal velocity (V_{max}) of AChE, and the activities of CarE and GST in *A. plutellae* were 0.22, 2.08, 4.60 and 0.45-fold as those in *P. xylostella*, respectively. The bimolecular rate constants (K_i) of AChE to methamidophos, dichlorvos and carbofuran in *A. plutellae* were 14.7, 10.5 and 26.0-fold as those in *P. xylostella*, respectively. High inhibition of AChE was found in both species when being incubated with insecticides at high temperature, especially in *A. plutellae*. The results indicated that the high susceptibilities to organophosphates and carbametes in *A. plutellae* were related to its high sensitivity of AChE to the insecticides, and the oxidative metabolism might be more effective in tolerance to insecticides than non-oxidative metabolism in *A. plutellae*. In addition, the causes of the intrinsic differences in insecticide selectivity in the two species were also discussed.

Key words: *Apanteles plutellae*; *Plutella xylostella*; insecticide susceptibility; acetylcholinesterase sensitivity; detoxification enzyme

1 INTRODUCTION

Indiscriminate use of insecticides had eliminated the parasitoids and predators which, to some extent, contributed to reducing the pest population in the past. As a result, the pest problem has been exacerbated and efforts are now being made to search for alternative control measures. Biological control, integration of chemical and biological control system for arthropods and application of integrated pest management-compatible pesticides have received more and more attention in pest management programs. It had been reported that satisfactory control of the diamondback moth *Plutella xylostella* (L.) was achieved using *Apanteles plutellae* Kurdjumov (Hymenoptera: Braconidae), a larval parasitoid of the diamondback moth (Lepidoptera: Yponomeutidae), in combination with compatible agents such as *Bacillus thuringiensis* (Chilcutt and Tabashnik, 1999). It was identified that *A. plutellae* had the greatest control potential among the parasitoids recorded on the diamondback moth (Talekar and Shelton, 1993). And there were some works about the susceptibility to insecticides in *A. plutellae* (Mani and Krishnamoorthy, 1984; Chiang and Sun, 1991).

The dissimilarities of detoxification enzymes existed in the predatory mite *Amblyseius fallacies* and its herbivorous prey *Tetranychus urticae* might be related both to differing adaptations to plant allelochemicals and to the higher respiration rate of the predator, and provided an insecticide selectivity favorable to the

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predator; hydrolytic and conjugating reactions appeared more important than oxidative pathways in imparting organophosphate resistance to these acarines (Mullin *et al.*, 1982). The activities of microsomal monooxygenase and glutathione S-transferase (GST) were far higher in *P. xylostella* (L.) than those in its parasitoids *A. plutellae* and *Diadegma semiclausum* Hellen, but the activities of carboxylesterase (CarE) were similar among the parasitoids and their host (Chiang and Sun, 1991). The synergistic effects of piperonyl butoxide (PB, inhibitor of mixed-function oxidase MFO) to acephate, profenofos, fenvalerate and permethrin were found in *A. plutellae*, and the synergism of PB to fenvalerate was especially significant (Feng and Wang, 1984). High level of resistance in *Anisopteromalus calandrae* was associated with increased activity of a malathion specific CarE (Baker *et al.*, 1998). There existed the correlated variations of acetylcholinesterase (AChE) sensitivity between *A. plutellae* and its host *P. xylostella* by the field monitoring (Wu *et al.*, 2000; Wu *et al.*, 2002), and the resistance to organophosphates and carbamates was associated with the insensitive AChE in *A. plutellae* (Wu *et al.*, 2002). However, the research on the insecticide susceptibility and biochemical mechanism in parasitoids were still sparse. The synergisms of PB and triphenyl phosphate (TPP, inhibitor of CarE) to avermectins, fipronil and chlorfluazuron, and the synergism of diethyl maleate (DEM, inhibitor of GST) to insecticides in parasitoids could not be found in the reports. The present study was made to determine the synergistic effect of enzyme inhibitor on insecticides in *A. plutellae* and compare the insecticide susceptibility and enzymetic characteristics in *A. plutellae* with its host *P. xylostella*.

2 MATERIALS AND METHODS

2.1 Chemicals

Piperonyl butoxide (PB), triphenyl phosphate (TPP), diethyl maleate (DEM), eserine, bovine serum albumin, reduced glutathione (GSH), 1-chloro-2, 4-dinitrobenzene (CDNB) and acetythioncholine (ATCh) iodide were obtained from Sigma Chemical Co., Ltd.. The 1-naphthyl acetate (α-NA) and other chemicals were of analytical grade. Technical grade insecticides provided by the manufactures were used in this study, except for fipronil and chlorfluazuron which were of commercial formulation. Their sources are: methamidophos (90% pure) from Shangming Insecticide Factory, China; carbofuran (98% pure) from Fuan Insecticide Factory, China; acephate (90% pure), dichlorvos (92.5% pure) and *bata*-cypermethrin (90% pure) from Sanonda Co., Ltd., China; fenvalerate (96% pure) from Sumitomo Chemical Co., Ltd., Osaka, Japan; cypermethrin (91.4% pure) from Changzhou Insecticide Factory, China; avermectins (95.7% pure, $B_1a \geqslant 80\%$) from North China Pharmaceutical Group Corporation Aino Co., Ltd., China; chlorfluazuron (5% EC) from Ishihara Co., Ltd., Japan; fipronil (Regent, 5% SC) from Rhone-Poulenc AG, France; *Bacillus thuringiensis* var. *kurstaki* 8010 (16 000 IU/mg, WP) from Biopesticide Factory, Fujian Agriculture and Forestry University, China.

2.2 Insects and Bioassays

P. xylostella and *A. plutellae* were collected from commercial vegetable district in Jianxin, Fuzhou, Fujian, China, and reared in an environmental chamber at 25℃ under a photoperiod of 16L∶8D. The third instar larvae of F_1 progeny of *P. xylostella* and adults of newly emerged *A. plutellae* from the same group of *P. xylostella* were used in the experiments. Bioassays were conducted in an environmental chamber at 25℃.

Vial residual film was used for the bioassay of *A. plutellae*, following the method of Chiang and Sun (1991). Acetone solution of insecticide was used to coat vial (1.5 cm diameter, 10 cm length). For calculating 1 h and 9 h mortality, adults of newly emerged *A. plutellae* were introduced into the vial and left in contact with the insecticide for 1 h. Knock-down number (for pyrethroids) or mortality (for other insecticides) was recorded immediately. Then, the treated insects were subsequently moved to a clean vial, provided with 15% honey, and the mortality was recorded 8 h later. While 24 h and 48 h mortalities were calculated after *A. plutellae* were left in contact with insecticides for 24 and 48 h, respectively. The definition of knock-down was that the adults of *A. plutellae* could not stand after being treated with pyrethroids. The definition of death was that the adults could not respond to a pencil tip prodding. At least 4 replicates were made per data. In the control only acetone was used. For calculating LC_{50}, a series of five concentrations plus an untreated control were tested, and each concentration was replicated three times, with 10 individuals of *A. plutellae* per replicate. Experiments were done with 150 adults per test data of LC_{50}. The residual film of Bt was prepared with water.

The mixed solution of insecticide and synergist (V∶V = 1∶1) was used to produce the residual film. The ultimate concentration of PB, TPP and DEM was 100 mg/L, and the concentration of synergists resulted in no mortality in the adult of *A. plutellae* when the synergists were used alone.

The leaf-dipping technique was adopted in bioassay of *P. xylostella* (Wu *et al.*, 2002). The data were obtained by recording the mortality 72 h after treatment in case of chlorfluazuron, or 48 h after treatment for all other insecticides.

2.3 Enzyme assays

Whole bodies of the 4th instar larvae of *P. xylostella* and adults of the parasitoid *A. plutellae* were used in the biochemical experiments, except only heads of larvae were used in the study of AChE of *P. xylostella*. *P. xylostella* and the parasitoid were homogenized in 0.066 mol/L sodium phosphate salt buffer, pH 7.8 (for AChE), pH 7.4 (for GST) and pH 7.0 (for CarE), and then the homogenate were filtered through glass wool. The filtrate was collected, and centrifuged at 1 500 × g (for AChE and CarE) or 10 000 × g (for GST) at 4℃ for 15 min. The buffer used in preparation of AChE contained 1% (V/V) Triton X-100. The buffer used in preparation of GST contained 4 mmol/L GSH. The supernatants were used as enzyme solutions for measuring activity and kinetic parameters of enzyme. Three samplings of insect were made for each assay. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as standard.

AChE activity was determined by the method of Ellman *et al*. (1961), with acetythiocholine (ATCh) as substrates in the presence of DTNB in phosphate buffer, pH 7.8 at 25℃, through measuring the optical density at 412 nm. CarE activity toward 1-naphthyl acetate (α-NA) was measured by the method of van Asperen (1962) with some modification, in phosphate buffer, pH 7.0 at 37℃, through measuring the optical density at 600 nm. The reaction mixture contained 4×10^{-4} mol/L eserine. For GST determination, the procedure of Habig (1981) was adopted with 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate in phosphate buffer, pH 7.4 at 25℃, by recording absorbance changes at 340 nm for 3 min.

2.4 Determination of kinetic parameters

2.4.1 K_m and V_{max} assays: The apparent Michaelis-Menten constant (K_m) and maximal velocity (V_{max}) for ATCh were determined from enzymatic activity measures at different substrate concentrations (from 5×10^{-7} mol/L to 1.0×10^{-2} mol/L), and the values were obtained from the Lineweaver-Burk plots.

2.4.2 K_i assays: The biomolecular rate constant (K_i) of AChE was estimated following the method of Aldridge (1950). Briefly, AChE was incubated with the inhibitor for various times before tipping the inhibition mixture into a solution of ATCh to measure residual activity. Assays were all carried out in triplicate in the presence or absence of inhibitor.

2.4.3 Effects of incubating temperature on the K_i: *A. plutellae* and *P. xylostella* were raised at 25℃, and F_2 progeny of the 4th instar larvae of *P. xylostella* and adults of *A. plutellae* from the same group of its host were used. The enzymes were incubated with dichlorvos at 37, 25, 5℃ respectively for various times before tipping the inhibition mixture into a solution of substrates to measure residual activity. The reactive temperatures of enzyme and substrate were 37, 25, 5℃, correspondingly.

2.5 Inhibition of synergists on enzyme activity *in vivo*

Two concentrations of PB, TPP and DEM for producing residual film of tube were 100 and 500 mg/L. The enzyme activities were detected 1 h after the adults of *A. plutellae* were introduced into the tube covered

Table 1 Selectivity of insecticides on the field *A. plutellae* and *P. xylostella* in Fuzhou

Insecticides	*A. plutellae*				*P. xylostella*	
	LC_{50} (95% FL) (mg/L) (24 h)	Slope ± *SE*	LC_{50} (95% FL) (mg/L) (48 h)	Slope ± *SE*	LC_{50} (95% FL) (mg/L) (48 h)	Slope ± *SE*
Methamidophos	12.59 (10.59 – 14.96)	3.90 ± 0.78	5.02 (4.27 – 5.92)	4.03 ± 0.44	1 556.47 (1 230.73 – 1 968.55)	2.29 ± 0.33
Acephate	189.93 (165.00 – 218.54)	4.81 ± 0.75	125.31 (106.24 – 148.22)	4.29 ± 0.84	1 175.20 (973.00 – 1 419.43)	3.08 ± 0.56
Fenvalerate	251.75 (213.78 – 296.46)	4.16 ± 0.98	155.41 (127.49 – 189.44)	3.88 ± 1.07	1 975.00 (1 605.35 – 2 429.84)	2.71 ± 0.24
Cypermethrin	195.63 (168.44 – 227.21)	4.34 ± 0.57	115.94 (97.29 – 138.06)	4.45 ± 0.73	1 566.37 (1 232.54 – 1 989.74)	2.26 ± 0.15
Avermectin	12.22 (10.28 – 14.52)	4.06 ± 0.79	6.94 (5.83 – 8.27)	3.59 ± 0.74	4.88 (3.97 – 6.00)	2.73 ± 0.20
Fipronil	0.78 (0.63 – 0.96)	3.00 ± 0.27	0.17 (0.14 – 0.21)	3.48 ± 0.73	2.89 (2.44 – 3.42)	3.76 ± 0.26
Chlorfluazuron	–	–	> 100	–	6.29(4.88 – 8.10)	2.40 ± 0.26
Bt	–	–	> 1 000	–	356.05(298.09 – 436.02)	2.91 ± 0.27

with the residual film (zero mortality); acetone was used in the control.

3 RESULTS

3.1 Susceptibility to insecticides in *A. plutellae* and *P. xylostella*

According to 48 h mortalities of *A. plutellae* and *P. xylostella*, organophosphates, carbamates, pyrethroids, avermectin and fipronil were high toxic to *A. plutallae*, but chlorfluazuron and Bt were not (Table 1). However, compared with LC_{50}s (calculated on 48 h mortalities) in *P. xylostella*, fenvalerate, cypermethrin, acephate, avermectin and fipronil were also low toxic to *A. plutellae* if the parasitoid was left in contact with the insecticides for only 1 h (Table 1 and 2). Different from being treated with organophosphates, carbamates, avermectins and fipronil, knock-down *A. plutellae* could recover from the treatment of pyrethroids (Table 2 and 3). LC_{50}s of 9 h were far higher than those of 1 h treated with pyrethroid in *A. plutellae* (Table 3). In our experiment, all of the parasitoids treated with fenvalerate and cypermethrin at 2 000 mg/L could not move their legs and antennae 30 min after treatment. However, all parasitoid treated with fenvalerate and 87% parasitoid treated with cypermethrin for 1 h could survive and walk normally at 8 h after being moved to an insecticide-free vial (Table 2).

Table 2 Insecticide susceptibility and synergistic effects of synergists on the field *A. plutellae* in Fuzhou

Insecticides	Dose (mg/L)	*n*	Mortality ± *SE* (1 h, %)	Mortality ± *SE* (9 h, %)
Methamidophos	225	50	69.7 ± 6.3	100
	50	55	0 a	5.0 ± 4.8 a
Methamidophos + PB	50 + 100	52	55.1 ± 8.9 b	93.7 ± 5.94 b
Methamidophos + TPP	50 + 100	57	7.4 ± 8.0 ac	21.1 ± 3.5 c
Methamidophos + DEM	50 + 100	58	15.2 ± 9.7 c	52.0 ± 8.2 d
Acephate	2 000	51	0	90.5 ± 9.0
	1 000	59	0	17.2 ± 13.1
Carbofuran	10	58	0 a	8.0 ± 7.9 a
Carbofuran + PB	10 + 100	59	50.1 ± 15.8 b	100 b
Carbofuran + TPP	10 + 100	56	17.9 ± 15.6 c	53.0 ± 20.0 c
Carbofuran + DEM	10 + 100	48	20.7 ± 11.6 c	71.4 ± 16.4 c
Fenvalerate	2 000	62	100 a	0 a
Fenvalerate + PB	2 000 + 100	64	100 a	66.3 ± 4.2 b
Fenvalerate + TPP	2 000 + 100	61	100 a	27.1 ± 3.5 c
Fenvalerate + DEM	2 000 + 100	63	100 a	16.8 ± 3.1 d
Cypermethrin	2 000	62	100 a	13.6 ± 4.1 a
Cypermethrin + PB	2 000 + 100	65	100 a	89.6 ± 8.6 b
Cypermethrin + TPP	2 000 + 100	60	100 a	37.1 ± 3.2 c
Cypermethrin + DEM	2 000 + 100	64	100 a	29.1 ± 5.1 c
Fipronil	75	59	0	47.6 ± 3.4
	50	77	0 a	0 a
Fipronil + PB	50 + 100	63	0 a	86.1 ± 3.9 b
Fipronil + TPP	50 + 100	68	0 a	22.4 ± 18.0 c
Fipronil + DEM	50 + 100	57	0 a	32.3 + 9.7 c
Avermectin	250	62	0	63.1 ± 4.3
	125	49	0 a	2.1 ± 3.6 a
Avermectin + PB	125 + 100	56	0 a	63.0 ± 15.7 b
Avermectin + TPP	125 + 100	62	0 a	20.1 ± 7.0 c
Avermectin + DEM	125 + 100	51	0 a	21.2 ± 4.2 c
Chlorfluazuron	500	59	0 a	1.6 ± 2.3 a
Chlorfluazuron + PB	500 + 100	56	0 a	0 a
Chlorfluazuron + TPP	500 + 100	57	0 a	0 a
Chlorfluazuron + DEM	500 + 100	57	0 a	0 a

Note: Means in the same column followed by different letters differ significantly in synergistic experiment ($P < 0.05$, Duncan's multiple range test) for each insecticide. The same below. Data in Table 2 was translated by arcsine transformation before the multiple test.

Table 3 Susceptibility to fenvalerate and cypermethrin in the field *A. plutellae* in Jianxin, Fuzhou

Insecticides	LC_{50}(95% CL)(mg/L)(1 h)	Slope ± *SE*	LC_{50}(95% CL)(mg/L)(9 h)	Slope ± *SE*
Fenvalerate	1 195.36 (1 065.59 – 1 340.94)	5.05 ± 0.67	7 452.27 (6 555.24 – 8 472.06)	5.16 ± 0.89
Cypermethrin	682.79 (614.79 – 758.38)	5.70 ± 0.79	2 407.96 (2 024.18 – 2 864.51)	3.47 ± 0.39

3.2 Synergistic effect of synergists on the insecticides

Synergistic effects of synergist on the insecticides in *A. plutellae* were showed in Table 2. Significant synergisms of PB, TPP and DEM on methamidophos, carbofuron, fenvalerate, cypermethrin, avermectins and fipronil were found except chlorfluazuron. The synergisms of DEM were far lower than those of PB, but slightly higher than those of TPP. The synergistic effects of PB were the highest. The recovery of knock-down parasitoid treated with pyrethroids could be inhibited by the synergists (Table 2).

3.3 Inhibition of synergists on enzyme activity *in vivo*

Low inhibition in CarE activity by PB and TPP and in GST activity by DEM was found when concentrations of PB, TPP and DEM were 100 mg/L *in vivo*. AChE activity could not be inhibited by PB, TPP and DEM. Strong inhibition could be found in CarE activity by PB and TPP and in GST activity by DEM when the concentrations of the synergists were 500 mg/L *in vivo* (Table 4).

Table 4 Inhibition of PB, TPP and DEM on the activity of AChE, CarE and GST in the field *A. plutellae in vivo*

Treatment	Concentration (mg/L)	AChE(± *SE*)		CarE(± *SE*)		GST(± *SE*, ×10^4)	
		ATCh	Inhibition (%)	α-NA	Inhibition (%)	CDNB	Inhibition (%)
PB	100	15.96 ± 0.30 a	0	530.23 ± 3.88 a	15.67	7.35 ± 0.34 a	0
TPP	100	16.02 ± 0.81 a	0	532.41 ± 4.09 a	15.33	7.43 ± 0.26 a	0
DEM	100	15.91 ± 0.46 a	0	602.80 ± 16.4 b	4.13	6.33 ± 0.66 b	14.45
CK	–	16.03 ± 0.46 a	–	628.78 ± 6.41 b	–	7.40 ± 0.32 a	–
PB	500	16.32 ± 0.26 a	5.67	436.86 ± 34.4 a	25.86	6.78 ± 0.34 a	5.04
TPP	500	16.48 ± 0.47 a	4.74	275.11 ± 15.0 b	53.31	7.00 ± 0.21 a	0
DEM	500	16.66 ± 0.55 a	3.79	554.60 ± 24.4 c	5.88	5.12 ± 0.43 b	28.29
CK	–	17.30 ± 0.15 a	–	589.23 ± 10.7 c	–	7.14 ± 0.18 a	–

Note: Activity units of AChE, CarE and GST are nmol·(mg protein·min)$^{-1}$.

3.4 Comparison of the activity of AChE, CarE and GST

The results including kinetic studies on hydrolysis of ATCh by AChE and the activity of CarE and GST in two species insects were summarized in Table 5. The K_m and V_{max} of AChE and the activity of CarE and GST in *P. xylostella* were 4.63, 0.48, 0.22 and 2.21-fold as high as those in *A. plutellae*.

3.5 Sensitivity of AChE to insecticides

The biomolecular rate constants (K_i) of AChE to methamidophos, dichlorvos and carbofuran, which provided a good measure of AChE sensitivity to inhibition, were far higher in *A. plutellae* than those in *P. xylostella* (Table 6). The inhibition of AChE by dichlorvos in *P. xylostella* and *A. plutellae* was increased significantly at high incubating temperature (37℃), especially in *A. plutellae* (Table 7).

Table 5 Comparison of the activities of AChE, CarE and GST between the field *A. plutellae* and *P. xylostella* in Fuzhou

Insects	AChE				CarE	Ratio	GST (×10^3)	Ratio
	K_m (×10^{-5})	Ratio	V_{max}	Ratio				
A. plutellae	6.84 ± 0.06	0.22	15.92 ± 0.16	2.08	630.35 ± 97.75	4.60	76.90 ± 3.75	0.45
P. xylostella	31.7 ± 1.37	1.0	7.65 ± 0.24	1.0	137.28 ± 23.51	1.0	170.06 ± 13.31	1.0

Note: Unit of K_m is mol/L. Activity units of AChE, CarE and GST are nmol·(mg protein·min)$^{-1}$.

Table 6 Comparison of the sensitivity of AChE to insecticides between the field *A. plutellae* and *P. xylostella* in Fuzhou

Insects	K_i ($\pm SE$) [(mol/L)$^{-1}$·min^{-1}]					
	Methamidophos ($\times 10^3$)	Ratio	Dichlorvos ($\times 10^4$)	Ratio	Carbofuran ($\times 10^5$)	Ratio
A. plutellae	2.64 ± 0.21	14.67	3.36 ± 0.39	10.50	2.25 ± 0.32	26.07
P. xylostella	0.18 ± 0.02	1.0	0.32 ± 0.05	1.0	0.14 ± 0.03	1.0

Table 7 Comparison of K_i of AChE to dichlorvos at the different incubating temperatures in field *P. xylostella* and *A. plutellae*

Temperature (℃)	*A. plutellae*		*P. xylostella*	
	K_i ($\times 10^4$) [(mol/L)$^{-1}$·min^{-1}]	Ratio*	K_i ($\times 10^3$) [(mol/L)$^{-1}$·min^{-1}]	Ratio*
37	10.23 ± 0.26 b	1.96	8.69 ± 0.60 b	1.60
25	5.31 ± 0.37 a	1.0	5.45 ± 0.19 a	1.0
5	4.92 ± 0.11 a	0.93	4.59 ± 0.13 a	0.84

* Ratio = K_i at 37℃ or 5℃ / K_i at 25℃, respectively.

4 DISCUSSION

Non-selective insecticides were high toxic to *A. plutellae* (Mani and Krishnamoorthy, 1984; Chiang and Sun, 1991). Generally speaking, parasitoids rarely could afford to survive under most effective control doses for the pest insects. However, *A. plutellae* could survive at the conventional control doses of avermectin, fipronil, fenvalerate, cypermethrin and acephate if the parasitoid was left in contact with the insecticides only for short time (1 h) according to the results in this study. In particular, recovery from the treatment of pyrethroids was found. LC_{50}s of 9 h for fenvalerate and cypermethrin were as high as 7 452 and 2 408 mg/L, respectively. It might be an important way to develop the insecticide resistance in *A. plutellae*. And these facts implied the potential of development of insecticide-resistant parasitoid for *P. xylostella* control in integrated pest management.

Feng and Wang (1984) reported that the LC_{50}s of fenvalerate, methamidophos and methomyl in *A. plutellae* were 0.75, 0.90 and 1.03 respectively (μg/test tube, mortality were recorded 2 h after treatment) by using residual film method. In this paper, the susceptibility to methamidophos was far higher than that to fenvalerate in *A. plutellae*. The results suggested that high tolerance to fenvalerate was developed in *A. plutellae* in Fuzhou.

The results about synergism indicated that the tolerance to insecticides in *A. plutellae* was related to detoxification of MFO, CarE and GST. PB showed the highest synergisms for all tested insecticides except chlorfluazuron. The highest synergisms of PB might be related not only to the inhibition of CarE which was found in this study, but also to the inhibition of MFO because PB is a specific inhibitor of MFO. Li *et al.* (2002) reported that the fenvalerate resistance in *A. plutellae* was related to MFO activity, but unrelated to CarE activity. Because MFO was an important broad spectrum detoxifying enzyme in insects, it was speculated from our results that the detoxification of MFO was more important than those of CarE and GST in the tolerance to the insecticides tested in *A. plutellae*. Various synergists were used to overcome the insecticide resistance in the field, especially the use of inhibitor of MFO. It suggested that it should be cautious to apply synergists to overcome the resistance of pest insects in field because synergists might decrease vitality of natural enemies.

The tolerance of herbivorous insects to insecticides was probably originated from the detoxification of the secondary substances of host plants, and the activity of detoxification enzyme could be introduced by the secondary substances of host plants (Terriere, 1984). The activity of enzymes in parasitoids, being without the selection pressure from the secondary substance in host plant, would not change as much as that in pest insects. The lack of genetic flexibility among the ecologically specialized parasitoids and the relatively low activity of preadapting detoxifying enzyme systems may restrict their evolution of insecticide resistance (Croft and Strikler, 1983). Because of indiscriminate use of insecticides in recent twenty years, however, it could be speculated that parasitoids had developed greatly their detoxifing enzyme system. Chiang and Sun (1991) reported that the activities of microsomal monooxygenase and GST in *A. plutellae* were 0.13 and 0.004 times as high as those in its host *P. xylostella*, and CarE activities were similar in the two species. Our results at present study showed that the activities of CarE and GST in *A. plutellae* were 4.60 and 0.45 times as high as those in *P. xylostella*. CarE and GST were important detoxifying enzymes in insects (Terriere, 1984). In contrast to the results reported by Chiang and Sun (1991), high activities of CarE and GST in the field population of *A. plutellae* in Fuzhou might be related to selective pressure of insecticides in

the field, and might play important roles in the tolerance to insecticides.

K_m values would mostly reflect the affinity of tenzyme for substrate. High activity of AChE might be related to its high affinity to substrate ACTh (Fournier and Mutero, 1994). Although *A. plutellae* and *P. xylostella* in Fuzhou had developed resistance to organophosphates and insensitive AChE (Wu *et al*., 2002), the modified AChE of *A. plutellae* showed very different kinetic parameters from its host *P. xylostella*. The characteristics in modified AChE would be affected by the environmental factors or habitation, although they were also related to the specific species of insects. High K_i of AChE in *A. plutellae* might be related to the lack of preadaptation and the mild selective pressure of insecticides on endoparasitoids protected by its hosts. High inhibition of AChE was found in the two species at high incubating temperature, which would lead to higher mortality under the spraying during hot season in the field and limited the development of organophosphate resistance in insects, in particular, in *A. plutellae*. It would be of no avail to parasitoids in its evolution of insecticide resistance.

High GST activity in *P. xylostella* might explain partly its high tolerance to methamidophos, acephate, fenvalerate, cypremethrin and fipronil, but the attempt to correlate the CarE activity with the insecticide susceptibility was unsuccessful in the two species. Because of rare survivor under most effective control doses for the pest insects, and the lack of genetic flexibility and the preadaptation, it could be speculated that milder selective pressure of insecticides on parasitoids, which could survive from the spraying, resulted in higher susceptibility to insecticides. Before the biochemical mechanism can be understood, more study of the causes of the intrinsic differences about insecticide selectivity is needed, especially on MFO characteristics and the specific targets of the insecticides in the two species.

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菜蛾绒茧蜂和小菜蛾对杀虫剂的敏感性及酶学特性的比较研究

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摘要：分别采用药膜法和浸叶法测定了菜蛾绒茧蜂 *Aparteles plutellae* 和小菜蛾 *Plutella xylostella* 对杀虫剂的敏感度。结果显示：有机磷、氨基甲酸酯、拟除虫菊酯类杀虫剂、阿维菌素和锐劲特对菜蛾绒茧蜂高毒，而抑太保和 Bt 为低毒，然而，短时间（1 h）接触常规防治剂量的锐劲特、氰戊菊酯、氯氰菊酯和乙酰甲胺磷对菜蛾绒茧蜂低毒。增效剂胡椒基丁醚（PB）、磷酸三苯酯（TPP）和马来酸二乙酯（DEM）对菜蛾绒茧蜂的甲胺磷、克百威、氰戊菊酯、氯氰菊酯、阿维菌素和锐劲特敏感性增效显著，但对抑太保无增效作用。PB 的增效作用显著高于 TPP 和 DEM。PB 和 TPP 对菜蛾绒茧蜂羧酸酯酶（CarE），以及 DEM 对谷胱甘肽 S 转移酶（GST）具显著的活体抑制作用，但 PB，TPP 和 DEM 对菜蛾绒茧蜂乙酰胆碱酯酶（AChE）无抑制作用。菜蛾绒茧蜂 AChE 的米氏常数（K_m）、最大反应速度（V_{max}）、CarE 和 GST 活性分别为小菜蛾的 0.22、2.08、4.60 和 0.45 倍，甲胺磷、敌敌畏和克百威对菜蛾绒茧蜂 AChE 的双分子速度常数（K_i）分别为对小菜蛾的 14.7、10.5 和 26.0 倍。酶与抑制剂反应温度增高将导致酶抑制率增高，尤其对菜蛾绒茧蜂 AChE 的抑制作用更为显著。上述结果表明，菜蛾绒茧蜂对有机磷和氨基甲酸酯类杀虫剂的高敏感性与其显著高的 AChE 敏感性有关，氧化代谢的解毒作用对菜蛾绒茧蜂耐药性的影响大于水解作用。此外，对小菜蛾和菜蛾绒茧蜂杀虫剂敏感性差异的毒理学原因进行了讨论。

关键词：菜蛾绒茧蜂；小菜蛾；杀虫剂敏感度；乙酰胆碱酯酶敏感性；解毒酶

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